A novel approach for the production of human recombinant BMP-2 for bone tissue engineering applications

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INTRODUCTION

Bone tissue engineering has been an increasing field of research during the last years. The ideal approach for a regenerative application would consist in the use of cells from the patient, scaffolding materials and differentiation growth factors. **Bone morphogenetic protein-2** (BMP-2) is one such growth factors with a strong ability to induce new bone and cartilage formation and has been used as a powerful osteoinductive component of several late-stage tissue engineering products for bone grafting. In this work, we aimed at obtaining high yields of human recombinant BMP-2 in a stable, pure and biologically active form by use of a new bacteria expression system that circumvents the disadvantages of conventional recombinant protein preparation methods and to perform a study of the stability conditions and the functionality of these peptides *in vitro* in human mesenchymal stem cells and C2C12 murine cell line.

MATERIALS & METHODS

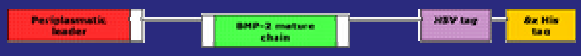


Fig. 1. The sequence coding for mature rhBMP-2 was cloned in a pET-25b vector and expressed in BL21DE3 *E. coli* strain. This vector permitted expression of recombinant protein into periplasm where ambient is permissive to the formation of cysteine bridges of folded protein.

rhBMP-2 was then purified by high affinity chromatography and size exclusion chromatography and tested in C2C12 cell line. This is a well-studied and stable model for testing the *in vitro* biological activity of recombinant BMPs.

Two variants of rhBMP-2 were produced: variant I containing two adjacent sites for protease cleavage in order to eliminate plasmid tags and variant II containing the protein with no additional cleavage sites.

For future bone biomedical applications!

Fig. 2. Expression of recombinant bacteria was performed in a **fermentor** allowing large yields of rhBMP-2, around 110mg/L.

RESULTS & DISCUSSION

Purification of rhBMP-2 by high affinity chromatography

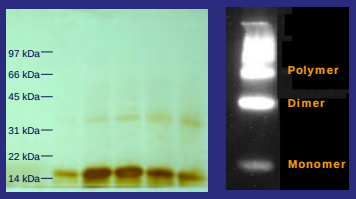


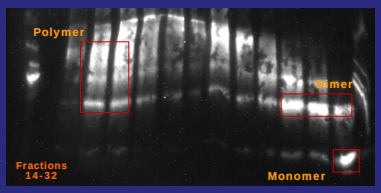
Fig. 3. Silver stained reduced SDS-PAGE reveals purification growth factor to up 95%

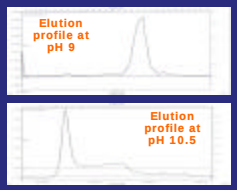
Fig. 4. Western-blot, non-reduced conditions permitted visualization of monomer, dimer and polymer fractions. An antibody against the 6x histidine tag was used.

rhBMP-2 was stabilized at pH 10 in PBS 0.5M L-arginine

Purification by size exclusion chromatography

Fig. 5. Size exclusion chromatography permitted partial separation of monomer, dimer and polymer fractions, as analysed by Western-blot.





Biological activity assays

MTS cytotoxicity assay

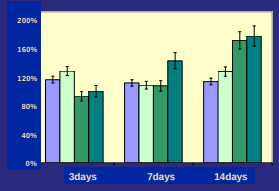


Fig. 6. MTS assay revealed no cytotoxicity of purified rhBMP-2

Morphology of human MSCs

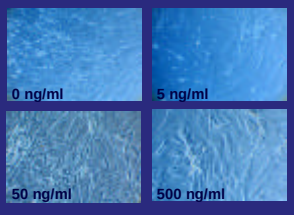
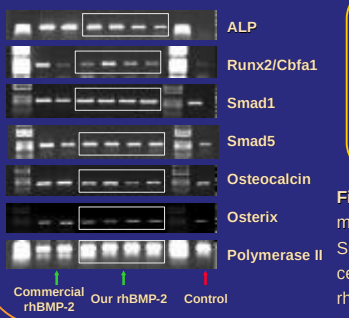


Fig. 7. Addition of 5-500ng/ml rhBMP-2 to human adipose mesenchymal stem cells resulted in changes of morphology

RT-PCR for specific markers



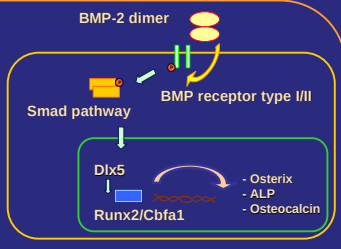


Fig. 8. RT-PCR shows increase of specific markers of osteogenic differentiation (ALP, Smad-5, runx2, osteocalcin) when C2C12 cells were stimulated with 500ng/ml of our rhBMP-2 stabilized at pH 10.

CONCLUSIONS

➢ The novel approach described herein shows to be a promising way for obtaining large amounts of partially purified rhBMP-2 which shows evidence of bioactivity, capable of inducing some markers of specific osteogenic (bone) differentiation and showing no relevant cytotoxicity.

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